**COMT × DRD4 Epistasis Impacts Prefrontal Cortex Function Underlying Response Control**

Sebastian Heinzel1,3,4,†, Thomas Dresler1,3, Christina G. Baehne1, Monika Heine1, Andrea Boreatti-Hümmer1, Christian P. Jacob1, Tobias J. Renner2, Andreas Reif1, Klaus-Peter Leisch1, Andreas J. Fallgatter1,5,‡ and Ann-Cristine Ehlers1,3,‡

1Department of Psychiatry, Psychosomatics, and Psychotherapy and 2Department of Child and Adolescent Psychiatry, Psychosomatics, and Psychotherapy, University of Würzburg, 97080 Würzburg, Germany 3Department of Psychiatry and Psychotherapy, University of Tübingen, 72076 Tübingen, Germany 4German Center for Neurodegenerative Diseases (DZNE), 72076 Tübingen, Germany

Address correspondence to Sebastian Heinzel, Psychophysiology and Optical Imaging, Department of Psychiatry and Psychotherapy, University of Tübingen, Calwerstrasse 14, 72076 Tübingen, Germany. Email: sebastian.heinzel@med.uni-tuebingen.de.

†This work is part of the dissertation of S.H.

‡A.J.F. and A.-C.E. contributed equally to the study.

The prefrontal cortex plays a major role in cognitive control, but it is unclear how single genes and gene–gene interactions (genetic epistasis) impact neural and behavioral phenotypes. Both dopamine (DA) availability (“inverted U-model”) and excitatory versus inhibitory DA receptor stimulation (“dual-state theory”) have been linked to important principles of prefrontal processing. Catechol-O-methyl-transferase (COMT; Val158Met) and DA D4-receptor (DRD4; 48 bp VNTR) genotypes were analyzed for effects on behavioral and neural correlates of prefrontal response control (NoGo-antiorization, NGA) using a Go–NoGo task and electroencephalography (114 controls and 181 patients with attention-deficit/hyperactivity disorder). DRD4 and COMT epistatically interacted on the NGA, whereas single genes and diagnosis showed no significant impact. Subjects with presumably relatively increased D4-receptor function (DRD4: no 7R-alleles) displayed an inverted U-relationship between the NGA and increasing COMT-dependent DA levels, whereas subjects with decreased D4-sensitivity (7R) showed a U-relationship. This interaction was supported by 7R-allele dose effects and mirrored by reaction time variability (non-significant after multiple testing correction). Combining previous theories of prefrontal DA functioning, neural stability at intermediate DA levels may be accompanied by the risk of overly decreased neural flexibility if inhibitory DA receptor function is additionally decreased. Our findings might help to disentangle the genetic basis of dopaminergic mechanisms underlying prefrontal (dys)function.

Current influential neural models of dopamine (DA) function within the PFC suggest that both DA levels and the ratio of stimulation of excitatory (D1-like receptor subfamily: D1 and D5) and inhibitory (D2-like: D2, D3, and D4) DA receptors play a pivotal role for PFC processing and functional outcome.

Specifically, (1) an inverted U-shaped relation between prefrontal DA levels and brain activation and task performance during cognitive control processes, as involved in working memory, has been repeatedly reported (for recent reviews, see Arnsten 2011; Cools and D’Esposito 2011). Moderate levels of D1-receptor stimulation have been shown to mediate stabilization of neural representations, neural tuning, and decrease in noise optimal for the online maintenance of information within the PFC (Seamans and Yang 2004; Vijayraghavan et al. 2007). However, too little or too much DA or D1-receptor stimulation, respectively, may be disruptive and impair these prefrontal processing features (Arnsten and Goldman-Rakic 1998; Bilder et al. 2004; Vijayraghavan et al. 2007).

Yet, neurobehavioral processes, such as cognitive control, are of multifactorial nature, often requiring both stable and flexible network characteristics (Cools and D’Esposito 2011). In this regard, (2) the “dual-state theory” postulates differential DA receptor type stimulation to underlie distinct energy barriers among prefrontal neuronal network patterns (Durstewitz and Seamans 2008). While a D1-dominated (high energy) network state favors cognitive stability, the D2-dominated state (low energy) is associated with cognitive flexibility among neural representational states.

Thus, depending on the nature of the task components, an imbalanced ratio of D1-like to D2-like receptor stimulation may bias prefrontal processing dynamics affecting cognitive performance, which may partly underlie deficits in attention and executive function in ADHD and schizophrenia (Durstewitz and Seamans 2008; Kehrer et al. 2008; Rolls et al. 2008). However, the precise interaction between DA levels and DA receptor type stimulation on prefrontal processing is still largely unclear. Thus, investigating prefrontal processes with regard to the impact and (epistatic) interaction of these 2 factors, as indicated by functional gene variants, may shed light on the (dys)regulation of dopaminergic processing underlying prefrontal functions such as cognitive control. Two candidate genes, which we hypothesized to exert such epistatic effects on prefrontal processing and behavioral...
outcome, code for catechol-O-methyltransferase (COMT) and the DA D4-receptor (DRD4).

The enzymatic degradation of DA by COMT (Karoum et al. 1994) is influenced by a single nucleotide polymorphism (SNP, Val158Met) causing a substitution of valine (Val) with methionine (Met) resulting in 3–4-fold reduced enzymatic activity and, therefore, increased baseline synaptic DA (Chen et al. 2004). In healthy subjects, prefrontal function is modulated by COMT-dependent DA levels, which maps to an inverted U-curve with best working memory performance at intermediate DA levels (Meyer-Lindenberg et al. 2005). However, genetic associations of Val158Met with ADHD diagnosis have been inconsistent (Gizer et al. 2009).

DA binding to D4-receptors (D2-like type) exerts inhibitory effects on neuronal and PFC activity (Yuen and Yan (2009)) balancing GABAergic inhibition and glutamatergic excitation via precise tuning mechanisms (Seamans, Gorelova et al. 2001). D4-receptor sensitivity has been shown to differ between the DRD4 variants, 4-repeats (4R) and 7-repeats (7R) of 48 bp, respectively. The 7R variant has a 2-fold decreased D4-receptor sensitivity compared with 4R (Asghari et al. 1995). Decreased D4-receptor function has been linked to impulsivity and novelty seeking (Ebstein et al. 1996; Avale et al. 2004), and the 7R-allele has been shown to be associated with ADHD [OR: 1.33, 1.15–1.54; (Gizer et al. 2009)].

The present study used an "Imaging Genetics" approach (first described in Fallgatter, Jatzke et al. 1999) to investigate statistical main effects and COMT × DRD4 epistasis on neurophysiological and behavioral correlates of cognitive response control in healthy controls and adult patients with ADHD.

Cognitive response control is one of the frontal lobe executive functions frequently disturbed in ADHD that has been suggested as a neurocognitive endophenotype of the disorder (Slaats-Wijlen et al. 2003). We used a reliable endophenotypic marker of prefrontal functioning, reflecting neural correlates of both response inhibition and execution in a Go–NoGo test situation [NoGo-antiorization (NGA; Fallgatter and Strik 1999)]. The NGA is a topographic event-related potential (ERP) parameter quantifying the brain’s electrical field frontализation during motor inhibition (NoGo, when compared with response execution: Go). Validated as a neurophysiological index of cognitive response control, the NGA reflects “NoGo” activation of the medial PFC (anterior cingulate cortex, ACC) (Fallgatter et al. 2002). The NGA and the electrical field frontализation during NoGo trials were shown to be reduced in schizophrenia and ADHD compared with healthy controls, reflecting diminished activation of the medial PFC in these patients (Fallgatter and Muller 2001; Fallgatter et al. 2003, 2005). Behavioral Go–NoGo performance can be quantified by the mean Go reaction time (Go-RT), its intra-individual variability (i.e. standard deviation, Go-RT SD), as well as response error rates. Particularly an increased Go-RT SD has been shown in patients with ADHD and linked to altered catecholaminergic processing (Castellanos et al. 2005). Functionally, increased mean Go-RTS as well as Go-RT SD may, as an adaptation strategy, allow for a decrease in response error rates; alternatively, an increased Go-RT SD has been discussed to reflect lapses in attention or an impaired “state regulation”, possibly due to cortical under-arousal (Klein et al. 2006).

Endophenotypic differences in cognitive response control (Sonuga-Barke 2002), decreased D4-receptor sensitivity (Faraone et al. 2005), and catecholaminergic dysfunction (Prince 2008) have been implicated in the pathophysiology of ADHD. Motivated by these findings, we investigated differences in genetic main and epistatic effects on neural and behavioral response control between healthy controls and ADHD patients. In order to determine the universality of our findings, we investigated a combined sample of healthy controls—with theoretically “normal” PFC DA metabolism—and ADHD patients, with putatively deviant PFC DA metabolism and an assumed DA-dependent dysregulation of PFC function.

Previously, our group showed gene main effects of tryptophan hydroxylase 2 (TPH2) and DA transporter (DAT1) on the NGA and Go and NoGo electrical fields, respectively, in healthy controls and adult ADHD patients (Baehne et al. 2009; Dresler et al. 2010). While adjusting our statistical tests to accommodate for this multiple testing situation, the present study aimed to investigate the hypothesis of COMT × DRD4 epistasis on neural and behavioral response control. We hypothesized that reduced neural flexibility due to decreased D4-receptor function (DRD4 7R genotype) would impact cognitive response control depending on prefrontal DA levels, specifically in the presence of intermediate DA levels/ D1-stimulation (COMT Val/Met genotype) fostering rigid and stable neural representations.

Materials and Methods

Participants

One hundred and eighty one adult ADHD patients and 114 healthy controls without history of psychiatric or neurological disorders were included in the study: ADHD patients as well as healthy controls were diagnosed by experienced psychiatrists using the Structured Clinical Interview for DSM-IV (SCID-I) (Wittchen et al. 1997). Participants were of Caucasian origin and recruited via the in- and outpatient facilities of the Department of Psychiatry, Psychosomatics, and Psychotherapy, University of Würzburg. All participants gave written informed consent. Patients and controls were currently not taking any psychotropic medication (89.5% of patients were naïve for ADHD medication and 10.5% were off medication for at least 3 days prior to testing). Further exclusion criteria were age below 18 and above 60 years and IQ below 90. The sample partly overlaps with 2 of our previous studies (Baehne et al. 2009; Dresler et al. 2010).

All participants were stratified according to the COMT Val158Met genotype (Val/Val, Val/Met, and Met/Met) and DRD4 48 bp VNTR genotype (no 7 repeat allele (no 7R), at least one 7 repeat allele (7R)). Genotype frequencies and descriptive statistics are displayed in Table 1. DRD4 genotype frequencies did not differ between diagnostic groups (χ² = 0.77, P = 0.38), and allele frequencies were in Hardy–Weinberg equilibrium (HWE) (controls: χ² = 0.73, P = 0.69 and ADHD: χ² = 0.01, P = 0.93). COMT genotype frequencies did not differ between diagnostic groups (χ² = 0.73, P = 0.69). COMT Val158Met allele frequencies were in HWE for controls (q² = 0.55, P = 0.46), but not for ADHD patients (q² = 4.01, P = 0.045).

Handedness, age distribution, and gender ratios did not differ between diagnostic groups or genotypes (P > 0.1). IQ scores were assessed using the MWT-B (Lehrl 2005), which measures crystallized verbal intelligence (for 2 patients no IQ data were available). For IQ scores, a 2 × 2 × 3 analysis of variance (ANOVA) yielded a significant effect for diagnosis (F₁,1272 = 15.83, P < 0.01, q² = 0.055), with higher scores for controls. ADHD patients had higher scores on the retrospective assessment of ADHD symptom severity in childhood [Wender Utah Rating Scale (WURS-k)] (Retz-Junginger et al. 2002) (F₁,272 = 189.53, P < 0.001, q² = 0.407; for 7 patients the WURS-k score was missing). COMT and DRD4 genotype, epistatic interactions, or interactions with diagnosis had no significant influence on WURS-k scores. 65.5% of the patients were diagnosed with the combined, 26.0% with the inattentive, and 8.5% with the hyperactive/
impulsive subtype of ADHD (for 8 patients, subtype diagnosis was missing). Subtype distribution did not differ between DRD4 and COMT genotype groups (P > 0.1).

49.2% of the ADHD patients had a current psychiatric comorbid axis I disorder as evaluated with the SCID-I (see Supplementary Material for further details). Furthermore, 82 ADHD patients (44.3%) and 19 controls (16.7%) were daily tobacco smokers (χ² = 16.49, P < 0.001).

The study was in accordance with the latest version of the Declaration of Helsinki and approved by the Ethics Committee of the University of Würzburg.

Genotyping

Genomic DNA was extracted from whole blood. Genotyping was performed as described previously for DRD4 48 bp VNTR (Ebstein et al. 1996) and COMT Val158Met (Egan et al. 2001), respectively, using polymerase chain reaction, enzymatic digestion, and gel electrophoresis. Further details on protocols are available upon request.

Electrophysiological Investigation

The electroencephalography (EEG) experiment was a Go–NoGo task (an OX version of the continuous performance test), which required participants to respond or to inhibit button responses to sequentially presented letter stimuli. Specifically, participants had to quickly respond when the letter O was directly followed by the letter X and to otherwise inhibit the response. Performance speed and accuracy were emphasized equally in the task instruction. After a short training session, the task comprised 400 letters (114 O = primer condition, 57 X following an O = Go condition, 57 other letters following an O = NoGo condition, and 172 letters not following an O = distractors), each presented for 200 ms with a stimulus-onset asynchrony of 1850 ms (total duration of the task: 13 min).

During the Go–NoGo task, a continuous EEG was recorded from 21 scalp electrodes placed according to the International 10/20 System. EEG signals were amplified by a 32-channel DC amplifier and recorded using "Vision Recorder" (Brain Products, Munich, Germany) with a bandpass filter (0.1–100 Hz) and 1000 Hz A/D rate. The recording reference was placed between Fz and Cz and the ground electrode between Fpz and Fz. All electrode impedances were kept below 5 kΩ.

Data Analysis

Electrophysiological data analyses were conducted using "Vision Analyzer" (Brain Products). After offline bandpass filtering (0.1–70 Hz), data were re-referenced to an average reference and corrected for artifacts (Supplementary Material). Artifact-free epochs with correct behavioral responses were segmented and averaged to Go and NoGo ERPs. Two-dimensional area centroids of P300 field maps (amplitude-weighted "centers of gravity" of the brain electrical field) were calculated for Go and NoGo conditions using individual P300 latencies at Pz (Go) and Cz (NoGo), respectively; P300 peaks were defined as the most positive deflection within 275–530 ms poststimulus. Individual centroids were localized on an anterior–posterior axis of a coordinate system resulting from the planar projection of the electrode array onto a rectangular grid. Positional centroid values were between 1 (Fpz) and 5 (Oz), and thus smaller values indicate a more anterior localization. Finally, the NGA was calculated individually as the difference between Go and NoGo centroids (Fallgatter and Strik 1999). Additionally, baseline-corrected Go and NoGo P300 peaks were exported for further (traditional) ERP analyses (baseline period: −100 to 0 ms pre-stimulus).

Source Localization

In order to identify brain regions significantly contributing to NGA differences between genotype groups, we utilized the standardized low-resolution brain electromagnetic tomography (sLORETA) software (http://www.uzh.ch/keyinst/loreta.htm) (Pascual-Marqui 2002). sLORETA is a weighted minimum norm inverse solution used to compute statistical maps from scalp potentials, indicating the location of underlying neural sources (Pascual-Marqui et al. 2002). Voxel-based sLORETA images were compared between 6 genotype groups (combined sample) using time points of maximal differences in scalp topography (Ehls et al. 2011). Genotype group differences between these maps were then statistically analyzed.

Statistical Analysis

Statistical analyses were performed using SPSS 17.0. Since the present sample largely overlaps with 2 of our previous studies investigating main effects of 2 SNPs within the TPH2 gene (Bachne et al. 2009) and 1 variable number of tandem repeats polymorphism of the DAT (SCL6A3; DAT gene) (Dresler et al. 2010), we used a Bonferroni correction for multiple testing considering the 5 investigated genetic polymorphisms (including COMT and DRD4). Therefore, the significance level was set to P < 0.01 for analyses of gene main and epistasis effects on neural (NGA) and behavioral measures. For analyses of genotypes impacting NGA and behavioral measures (mean Go–RT and Go–RT SD), 2 × 2 × 3 ANOVAs were conducted, comprising the between-subject factors "diagnosis," "DRD4," and "COMT." For conventional analysis of P300 amplitudes, similar ANOVAs were applied, additionally comprising the within-subject factors "condition" (Go vs. NoGo) and "electrode position" (Cz and Pz). Since post hoc analyses aimed to identify effects already indicated by ANOVA models corrected for comparison of multiple gene variants, post hoc tests and (exploratory) sLORETA tests were calculated using 2-tailed t-tests for independent samples, which were uncorrected for multiple testing (P < 0.05). Equality of variances was tested by means of Levene’s test. According to Kolmogorov–Smirnov’s Z-statistic, all data were normally distributed except Go–NoGo task performance error data (P < 0.01), for which Mann–Whitney U tests and Kruskal–Wallis tests were applied for between-group comparisons. Tests for quadratic relationships were performed using polynomial trend tests (P < 0.05).
Figure 1. (a) Intra-individual variability in Go-RT (Go-RT SD) in COMT genotype subgroups of DRD4 “no 7R”-carriers of the combined sample (ADHD patients and controls). The spline curve through mean Go-RT SD values displays the quadratic relationship of Go-RT SD between COMT genotypes depending on DRD4 × Go-RT SD were observed (P < 0.05) and increased Go-RT SD (F(45.39) = 460.34, P < 0.001, \( \eta^2 = 0.84 \)) compared with controls. Additionally, patients made more omission errors (U = 8044.0, Z = −3.46, P < 0.01) and commission errors (type 1) after primers and distractors (U = 7858.0, Z = −3.81, P < 0.01). The number of successful response inhibitions during NoGo trials (commission error type 2) did not differ between controls and ADHD patients (U = 10 247.5, Z = −0.181, P > 0.1).

No main effects of DRD4 or COMT genotype on Go-RT and Go-RT SD were observed (P > 0.1). Epistatic interactions of DRD4 × COMT on Go-RT (F(2,283) = 3.13, P = 0.045, \( \eta^2 = 0.022 \)) and Go-RT SD (F(2,283) = 3.62, P = 0.028, \( \eta^2 = 0.025 \); Fig. 1a,b) were numerically present, but did not reach the predefined significance level after correcting for multiple testing. Also, ADHD diagnosis did not explain further variance of gene main or epistatic effects (P > 0.3) (for separate analyses of diagnostic groups, see Supplementary Material). The number of COMT Met alleles and Go-RT SD followed quadratic relationships depending on DRD4 genotype. Subjects (combined sample) without 7R-alleles displayed a U-relationship (F(1,174) = 8.91, P = 0.003), whereas 7R-carriers showed a marginally significant inverted U-relationship (F(1,115) = 3.79, P = 0.054). Exploratory analyses of DRD4 7R-allele dose effects on Go-RT SD supported this finding. Descriptively, homozygous carriers of DRD4 7R showed a more pronounced inverted U-shape than 7R heterozygotes. Specifically, 7R homozygotes with COMT Met/Met genotype (n = 9) exhibited decreased Go-RT SD compared with corresponding 7R heterozygotes (n = 40) (Fig. 1c).

DRD4 genotype had no influence on omission or commission errors (P > 0.1). COMT genotype had no impact on commission errors (P > 0.1), but a significant effect on omission errors in controls (\( \chi^2 = 11.75, P = 0.003 \)). Here, Val/Met carriers made less omission errors compared with Val/Val (U = 550.0, P = 0.001) but not with Met/Met (U = 598.5, P = 0.037) carriers, whereas Val/Val and Met/Met did not differ significantly (U = 401.5, P > 0.1; Table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descriptive statistics of behavioral data (standard deviation in parentheses)</td>
</tr>
<tr>
<td>ADHD (n = 181)</td>
</tr>
<tr>
<td>COMT genotypes</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Met/Met</td>
</tr>
<tr>
<td>Commission</td>
</tr>
<tr>
<td>errors type 1</td>
</tr>
<tr>
<td>errors type 2</td>
</tr>
<tr>
<td>Go-RT</td>
</tr>
<tr>
<td>Go-RT SD</td>
</tr>
<tr>
<td>Controls (n = 114)</td>
</tr>
<tr>
<td>COMT genotypes</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Met/Met</td>
</tr>
<tr>
<td>Commission</td>
</tr>
<tr>
<td>errors type 1</td>
</tr>
<tr>
<td>errors type 2</td>
</tr>
<tr>
<td>Go-RT</td>
</tr>
<tr>
<td>Go-RT SD</td>
</tr>
</tbody>
</table>

Results

Behavioral Data

The ANOVA of Go-RT and Go-RT SD yielded a significant main effect for diagnosis. ADHD patients had marginally significantly longer Go-RT (F(1,283) = 6.04, P = 0.015, effect size \( \eta^2 = 0.021 \)) and increased Go-RT SD (F(1,283) = 14.64, P < 0.001, \( \eta^2 = 0.049 \)) compared with controls. Additionally, patients made more omission errors (U = 8044.0, Z = −3.46, P < 0.01) and commission errors (type 1) after primers and distractors (U = 7858.0, Z = −3.81, P < 0.01). The number of successful response inhibitions during NoGo trials (commission error type 2) did not differ between controls and ADHD patients (U = 10 247.5, Z = −0.181, P > 0.1).

No main effects of DRD4 or COMT genotype on Go-RT and Go-RT SD were observed (P > 0.1). Epistatic interactions of DRD4 × COMT on Go-RT (F(2,283) = 3.13, P = 0.045, \( \eta^2 = 0.022 \)) and Go-RT SD (F(2,283) = 3.62, P = 0.028, \( \eta^2 = 0.025 \); Fig. 1a,b) were numerically present, but did not reach the predefined significance level after correcting for multiple testing. Also, ADHD diagnosis did not explain further variance of gene main or epistatic effects (P > 0.3) (for separate analyses of diagnostic groups, see Supplementary Material). The number of COMT Met alleles and Go-RT SD followed quadratic relationships depending on DRD4 genotype. Subjects (combined sample) without 7R-alleles displayed a U-relationship (F(1,174) = 8.91, P = 0.003), whereas 7R-carriers showed a marginally significant inverted U-relationship (F(1,115) = 3.79, P = 0.054). Exploratory analyses of DRD4 7R-allele dose effects on Go-RT SD supported this finding. Descriptively, homozygous carriers of DRD4 7R showed a more pronounced inverted U-shape than 7R heterozygotes. Specifically, 7R homozygotes with COMT Met/Met genotype (n = 9) exhibited decreased Go-RT SD compared with corresponding 7R heterozygotes (n = 40) (Fig. 1c).

DRD4 genotype had no influence on omission or commission errors (P > 0.1). COMT genotype had no impact on commission errors (P > 0.1), but a significant effect on omission errors in controls (\( \chi^2 = 11.75, P = 0.003 \)). Here, Val/Met carriers made less omission errors compared with Val/Val (U = 550.0, P = 0.001) but not with Met/Met (U = 598.5, P = 0.037) carriers, whereas Val/Val and Met/Met did not differ significantly (U = 401.5, P > 0.1; Table 2).
NGA did not differ between ADHD patients and controls ($F_{1,283} = 0.56, P = 0.46$; ADHD: $0.62 \pm 0.43$ and controls: $0.67 \pm 0.36$; mean $\pm$ SD). Furthermore, no significant main effects of DRD4 and COMT genotype on the NGA were observed ($DRD4: F_{1,283} = 0.10, P = 0.76$ and $COMT: F_{2,283} = 0.06, P = 0.94$). However, $DRD4 \times COMT$ showed a significant interaction ($F_{2,283} = 6.21, P = 0.002, \eta^2 = 0.042$; Fig. 2a, b). Subjects lacking the DRD4 7R-allele exhibited an inverted U-relationship between the number of COMT Met alleles and the NGA ($F_{1,174} = 7.78, P = 0.006$), whereas DRD4 7R-allele carriers showed a marginally significant U-relationship ($F_{1,115} = 6.49, P = 0.012$). As diagnosis did not significantly impact the $DRD4 \times COMT$ interaction, its shape was similar in ADHD patients and controls (Supplementary Material). Post hoc analyses showed that DRD4 genotype impacted NGA in Val/Met ($t_{128} = 3.20, P = 0.002, \text{Cohen’s } d = 0.56$) and Val/Val ($t_{80} = -2.26, P = 0.03, d = 0.51$), but not in Met/Met carriers ($t_{81} = -0.90, P = 0.37, d = 0.21$) in the combined sample.

The profound impact of DRD4 and COMT epistasis on NGA was additionally supported by 7R-allele dose effects. Descriptively, in homozygous carriers of DRD4 7R, the NGA followed a more pronounced U-relationship with increasing Met alleles compared with 7R heterozygotes (Fig. 2c). In COMT Val/Met carriers, DRD4 7R homozygotes showed a significantly decreased NGA compared with 7R heterozygotes ($t_{47} = 2.46, P = 0.018, d = 0.93$).

Statistical results of genetic main and interaction effects on the NGA and behavioral measures remained largely unchanged after introducing “age” and “gender” as covariates in the analysis, as we expected from previous findings (Fallgatter, Mueller et al. 1999).

Complementing our findings of the genetic impact on the relative measure of the NGA, traditional ERP waveforms of Go and NoGo trials, respectively, as well as the corresponding topography, are shown for controls and ADHD patients in Figure 3a, b. Supporting findings of $COMT \times DRD4$ epistasis on the NGA, analyses of the Go and NoGo P300 amplitudes at
electrode positions Cz and Pz also showed a significant (at $P<0.05$, i.e. uncorrected for multiple testing) 4-fold interaction ($F_{2,283} = 3.24$, $P = 0.04$) between conditions (Go and NoGo), positions (Cz and Pz), DRD4 (7R and no 7R), and COMT (0, 1, and 2 Met alleles). Post hoc tests revealed that, for instance, "no 7R"-carriers exhibited an inverted U-relationship between P300 amplitudes and the number of COMT Met alleles (most pronounced for the NoGo condition and position Cz: $F_{1,114} = 4.16$, $P = 0.04$, quadratic trend test), which exactly mirrors the finding reported previously for the NGA. In contrast to that, for the group of 7R-carriers, the corresponding test showed a rather linear relationship ($F_{1,115} = 2.77$, $P = 0.099$; linear trend test). Again, similar to the NGA data, diagnosis had no additional impact on these genetic interactions ($P > 0.1$). However, (1) compared with controls, ADHD patients generally showed significantly lower P300 amplitudes in both task conditions at both positions (main effect diagnosis: $F_{2,283} = 19.36$, $P < 0.01$); (2) the reduction in P300 amplitude from Go to NoGo at Pz was more pronounced in ADHD patients ($1.59 \pm 2.49 \mu V$) compared with controls ($0.82 \pm 2.83 \mu V$; $t_{293} = 2.45$, $P < 0.05$); whereas (3) the amplitude increase from Go to NoGo at Cz was more pronounced in controls ($4.61 \pm 3.55 \mu V$ vs. $2.64 \pm 3.14 \mu V$; $t_{293} = 4.97$, $P < 0.001$) underlining the interaction of condition x position x diagnosis ($F_{1,283} = 8.63$, $P = 0.004$; see also Fig. 3a,b).

P300 amplitudes were negatively correlated with behavioral measures (Go trials at Pz: Go-RT: $r = -0.34$, Go-RT SD: $r = -0.27$ and NoGo trials at Cz: Go-RT: $r = -0.41$, Go-RT SD: $r = -0.52$, $P < 0.001$). NoGo-P300 values remained highly significantly correlated with behavioral measures in the 6 genotype subgroups, whereas correlations of Go-P300 values with Go-RT and Go-RT SD ranged between $r = -0.12$ (not significant) and $r = -0.60$ ($P < 0.001$) in the six genotype subgroups. For the NGA, smaller negative associations with behavioral measures were observed (Go-RT: $r = -0.12$, $P = 0.04$ and Go-RT SD: $r = -0.11$, $P = 0.07$).

**Source Localization**

Source localization revealed Brodmann area 6 (precentral gyrus) to significantly contribute to differences in NGA comparing $DRD4$ genotype groups in $COMT$ Val/Met carriers ($t_{120} = 3.58$, $P = 0.013$; Montreal Neurological Institute coordinates: $x = 35$, $y = -11$, $z = 40$; Fig. 4) and comparing $COMT$ Val/Met with Val/Val genotypes within $DRD4$ no 7R-carriers ($t_{120} = 3.98$, $P = 0.014$; supplementary motor cortex; $x = 25$, $y = -5$, $z = 70$). All other source localizations using genotype group comparisons did not reach statistical significance.

**Discussion**

The present data provide evidence for an epistatic interaction of $DRD4 \times COMT$ on neurophysiological correlates of prefrontal function underlying cognitive response control in adult ADHD patients and healthy controls. Prefrontal response control as indicated by the anterior shift of the brain electrical field during NoGo relative to Go trials (NGA) followed (inverted) U-shapes with increasing $COMT$-dependent DA levels, depending on $DRD4$ genotype. Genotype-dependent effects on the NGA could be localized in an explorative analysis to the right premotor and supplementary motor area. Mirroring the neural effects, $COMT$ and $DRD4$ epistasis showed similar (inverted) U-effects on the behavioral level (Go-RT and Go-RT SD), which were, however, no longer significant after strictly correcting for multiple testing. These results suggest that the function of D4-receptors depending on prefrontal DA levels has a profound impact on PFC processing. Moreover, the present results confirm our hypothesis that a genotype-dependent dysbalance of inhibitory (D4) and excitatory (D1; intermediate DA levels) DA receptor stimulation would decrease cognitive control function as indicated by the NGA and Go-RT SD. ADHD diagnosis had no significant impact on these epistatic interactions, and our findings may, thus, reflect basic principles of the genetic and dopaminergic regulation of PFC processing. The findings are discussed regarding the inverted U-model and the dual-state theory of prefrontal DA function as well as possible underlying neurobiological mechanisms.

Commonly, effects of varying DA levels have been investigated regarding actions of D1-receptors. For instance, D1-receptors have been shown to increase activity of PFC pyramidal neurons by enhancing N-methyl-D-aspartate (NMDA) receptor postsynaptic currents (Seamans, Durstewitz et al. 2001; Wang and O’Donnell 2001) and to increase feedforward GABAergic inhibition of pyramidal neurons reducing background activity (Durstewitz et al. 2000; Durstewitz and Seamans 2002). This may partly explain increasing signal-to-noise ratio (SNR) with increasing $COMT$-dependent DA levels (Winterer et al. 2006). Furthermore, for both DA concentration and D1-
receptor stimulation, an inverted U-shaped response function has been proposed, according to which too little or too much DA or D1-receptor stimulation, respectively, is disruptive and impairs functioning of the system (Arnsen and Goldman-Rakic 1998; Bildner et al. 2004; Vijayraghavan et al. 2007).

As a basis for the dual-state theory, effects of DA depend on the relative amount and efficiency of different DA receptor classes in target regions. Thus, the dual-state theory represents an extension of the inverted U-shaped model, which focusses on D1-stimulation and DA levels, respectively. While the precise mechanisms are not yet completely understood, DA-concentration dependency of D2-receptors or receptor-class differences in synaptic localization might impact the relative D1/D2-stimulation (Durstewitz and Seamans 2008). Thereby, neurophysiological effects may be mediated, which underlie the dual state. For instance, D4- and D1-receptors are expressed on pyramidal cells and GABAergic interneurons, where D4-receptors have been shown to attenuate both NMDA-mediated synaptic responses of pyramidal neurons and their inhibition via GABAergic interneurons (Seamans, Gorelova et al. 2001; Wang et al. 2003). Generally, D1- and D2-like (including D4) receptor function is closely interwoven in prefrontal systems, with partly antagonistic effects of both receptor types. It has, for example, been shown that D2/D4-receptor stimulation can truncate D1-mediated excitation in time, thereby acting as an inhibitory modulator of D1 action (Seamans, Gorelova et al. 2001). Moreover, relative activity of the 2 receptor classes seems to partly depend on the background dopaminergic tone and the strength of phasic (task-related) stimulation (Seamans and Yang 2004).

Our findings of an opposite effect of D4-receptor sensitivity (7R- vs. no 7R-carriers) in COMT Val/Met versus Val/Val carriers (Fig. 2) are in line with the above-mentioned physiological principles: Under conditions of intermediate DA availability (Val/Met), higher values of the NGA (and lower Go-RT and Go-RT SD) were observed in the DRD4 no 7R group, that is, subjects exhibiting relatively increased D4-receptor sensitivity. In this case, relatively increased D4-receptor functioning may provide optimal antagonism to (excitatory) D1 activity, resulting in optimal SNR and PFC processing. Under conditions of reduced DA availability (COMT Val/Val), however, higher NGA values were observed in subjects with a putatively reduced D4-receptor function (DRD4 7R). In terms of the above-mentioned model, this is also plausible as—under conditions of low DA availability and, thus, low D1-receptor stimulation—inhibitory effects of D4-receptor activation might be less beneficial for PFC functioning. In turn, low SNR present at low DA levels (Val/Val) (Winterer et al. 2006) may be enhanced when D4-receptors act less inhibitory (7R genotype) on interneuronal networks, thereby enhancing signal integration and the neural processing underlying the NGA (Fig. 2a). Thus, hypodopaminergic states (Val/Val) may be compensated by decreased D4 function, enhancing neural tuning in PFC circuits. Note that a significant impact of DRD4 genotype on NGA values was observed in both COMT Val/Val and Val/Met, but not in Met/Met carriers. Therefore, high SNR in hyperdopaminergic states (Met/Met) may attenuate the impact of DRD4 genotype on neurophysiological and behavioral measures of prefrontal response control. These findings and the dual-state theory are partly supported by a recent study of an interaction of COMT genotype and sulpiride, a selective DA D2-receptor blocker, on neural and behavioral correlates of error processing (Mueller et al. 2011). Here, COMT Val+ carriers (combined group of Val/Val and Val/Met) showed a relative decrease in error-related negativity (generated in the anterior midcingulate cortex) under sulpiride compared with Val+ under a placebo, whereas in Met/Met carriers, sulpiride had no significant impact. Behaviorally, these neural findings were mirrored by similar COMTx medication effects on posterror RT slowing (PES). Using a different EEG task and investigating gene–gene interactions, we detected similar neural (and behavioral) effects when comparing Val/Met carriers in our study with Val+ carriers investigated by Mueller et al. (2011). In these COMT groups, a decrease in (inhibitory) DA receptor function is accompanied with a decrease in neural activation (and task performance). However, our study separately investigating Val/Met carriers might allow for a more refined analysis and interpretation: Intermediate DA levels (Val/Met) are usually thought to be beneficial for neuronal tuning mechanisms underlying the inverted U-relationship of D1-receptor stimulation and stable neural representations as required, for example, for working memory (Seamans and Yang 2004; Vijayraghavan et al. 2007). According to the dual-state theory, stability of neural representations associated with a D1-dominated state (at intermediate DA levels) might be accompanied with the risk of decreased flexibility when D2-like receptor function is reduced. Here, DRD4 genotype may play an important role, as D2-like receptors have been implicated in flexible neural integration of new information (Durstewitz and Seamans 2008). Reduced NGA (and increased Go-RT and Go-RT SD) may, thus, be a result of an imbalanced ratio of D1/D2-like receptor stimulation present in COMT Val/ Met carriers with decreased D4-sensitivity (DRD4 7R). According to such an interpretation, reduced neural flexibility would result in less efficient transitions from neural Go to NoGo representations, thereby limiting performance, that is, increasing Go-RT and Go-RT SD (for example, due to a compensation strategy to prevent commission errors).

Importantly, cognitive response control is a multifactorial process that requires both cognitive stability and cognitive flexibility to be dynamically balanced (Cools and D’Esposito 2011). Thus, a balance of D1 and D2 states may be required for optimal neural processing and behavioral performance during cognitive response inhibition. Our results suggest that in Val/Met carriers (increased excitatory D1-stimulation) with a genotype-dependent decrease in inhibitory DA-receptor function (D2-like receptor family), this balance might be critically disturbed resulting in decreased NGA and, possibly to prevent commission errors during NoGo trials, an increase in response time and its variability (not significant after Bonferroni correction).

Neuroanatomically, the influence of DRD4 genotype on the NGA within COMT Val/Met carriers was localized to the right premotor and supplementary motor area (Brodmann area 6), which has been shown to be activated during response inhibition (Aron et al. 2007; Congdon et al. 2009). Moreover, these regions are strongly connected to the subthalamic nucleus projecting to the globus pallidus, which may inhibit the cortico-subthalamic program underlying the Go response (Aron and Poldrack 2006). The neural regulation of the cortico-subthalamic loop and fronto-striatal-thalamic networks mediating response inhibition behavior (Stevens et al. 2007) has been suggested to be modulated by genotype-
dependent DAT (Dresler et al. 2010; Cummins et al. 2011) as well as by COMT function (Congdon et al. 2009). Here, our results extend previous findings by showing that on the level of the right premotor and supplementary motor area, the epistatic interaction of COMT and DRD4 significantly impacts neural functioning of cognitive response control. However, other prefrontal structures, such as the inferior and middle frontal gyri, have been implicated to play an important role in response inhibition (Chikazoe 2010). Future imaging genetic studies should address the issue of a possibly differential impact of (dopaminergic) genotypes on neural processing within different brain regions involved in response inhibition. 

Previous studies investigating an interaction of DRD4 and COMT genotype on behavior and neural activation in healthy subjects were conducted using reward (Marco-Pallares et al. 2009; Camara et al. 2010) and performance monitoring paradigms (Kramer et al. 2007). These studies found no epistatic effect of DRD4×COMT (DRD4×521 C/T and COMT Val158Met), possibly due to small sample sizes and the systematic exclusion of heterozygous COMT Val/Met carriers.

While the present study aimed to investigate a genetic impact on the NGA as a relative measure (Go–NoGo) of response control processes linked to the ACC, COMT×DRD4 epistasis showed a similar but less pronounced effect on the level of position- and condition-dependent P300 amplitudes. Again, this epistatic interaction was not significantly modulated by ADHD diagnosis. Moreover, while the negative correlation of NGA and performance measures was weak (r = −0.12), P300 amplitudes underlying the NGA effect showed highly significant negative correlations with Go-RT and Go-RT SD with values between −0.27 and −0.52, substantiating the relevance of these neural measures for inhibitory control. In line with a meta-analysis on the Go and NoGo P300 in adult ADHD (Szüromi et al. 2011), ADHD patients showed reduced P300 amplitudes compared with controls, particularly in the NoGo condition. The significant interaction of diagnosis, condition, and electrode position (Fig. 3a,b) indicated altered (topographical) P300 characteristics in a patient group with putative problems in cognitive control and response inhibition. Taken together, the present findings suggest COMT×DRD4 epistasis to exert an impact on prefrontal processing independent of ADHD diagnosis, whereas in ADHD, additional neural alterations might be involved affecting P300 amplitudes and Go-NoGo task performance.

Some limitations of our study have to be considered. (1) Although the sample size is large for imaging genetic studies, it is relatively small regarding the multitude of (epi) genetic factors, which may also impact prefrontal processing. (2) The influence of other neurotransmitter systems modulating prefrontal processing such as glutamate, GABA, and serotonin was not investigated. (3) In ADHD patients, the criteria for HWE regarding COMT Val158Met were not met. This disequilibrium (P = 0.045) was due to a slightly increased number of COMT homozygotes in ADHD patients, which, however, should not affect the COMT×DRD4 epistasis (in COMT heterozygotes). (4) As common in this clinical population, 49.2% of the ADHD patients had a current comorbid disorder (see Supplementary Material for further details). While our sample of ADHD patients may, therefore, be representative, we cannot exclude the possibility that features of the comorbid disorders introduced some error variance in our data. However, introducing the presence of a comorbid disorder as a fixed factor had no significant impact on gene main or epistatic effects. (5) ADHD patients showed no statistical difference in NGA compared with healthy controls, which is in contrast to previous findings (Fallgatter et al. 2005). However, after excluding ADHD patients with any comorbid disorder, the remaining patients (n = 92) showed the expected decreased NGA compared with healthy controls (F201 = −2.09, P = 0.038, d = 0.29). Even the reduced sample of controls and ADHD patients (n = 206) showed the epistatic COMT×DRD4 interactions on the NGA (P = 0.016), Go-RT (P = 0.028), and a relatively increased epistatic effect on Go-RT SD (P = 0.006). (6) Due to the limited number of electrodes used in this study (21 scalp electrodes), the validity of our sLORETA findings may be reduced, and therefore comparison of (smaller) genotype groups might lack sufficient statistical power to additionally show genotype effects in inferior frontal gyrus and other frontal regions contributing to variance of the NGA. Therefore, our sLORETA results should be considered exploratory and require further validation.

Considering the interdependency of DA availability with specific DA receptor functions on SNR and network dynamics (stable vs. flexible) might help to disentangle neural mechanisms of prefrontal functioning. For instance, attention and executive function deficits in ADHD and schizophrenic spectrum disorders, which may partly emerge from imbalanced integration of DA signals via D1- and D2-like receptors (Durstewitz and Seamans 2008), might also have to be viewed in the light of (COMT-dependent) DA availability. Specifically, intermediate prefrontal DA levels (COMT Val/Met genotype) might represent a dopaminergic state in which prefrontal processing is increasingly vulnerable to decreased D4-receptor sensitivity (DRD4 7R-allele), which profoundly impacts prefrontal functioning underlying cognitive response control. Furthermore, our findings may provide important hypotheses for future imaging genetic studies investigating the dopaminergic regulation of prefrontal function as well as genetic association studies of ADHD and schizophrenia considering genetic epistasis.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding
This study was supported by the Deutsche Forschungsgemeinschaft (KFO 125 and SFB TRR 58 C4) and the Bundesministerium für Bildung und Forschung (BMBF 01GV0605).

Notes
The authors would like to thank T. Töpner, N. Steigerwald, N. Döring, J. Auer, and S. Herterich for excellent technical assistance and help with performing the genotyping. Conflict of Interest: None of the authors reported any biomedical financial interests or potential conflicts of interest.

References


Supplemental material

COMT x DRD4 epistasis impacts prefrontal cortex function underlying response control

Running title: COMT x DRD4 epistasis on prefrontal cortex function

Sebastian Heinzel, MSc1,2,3,†, Thomas Dresler, PhD1,2, Christina G. Baehne, MSc1, Monika Heine, MD1, Andrea Boreatti-Hümmper, MD1, Christian P. Jacob, MD1, Tobias J. Renner, MD4, Andreas Reif, MD1, Klaus-Peter Lesch, MD1, Andreas J. Fallgatter, MD2,* Ann-Christine Ehlis, PhD2,*

1Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Germany
2Department of Psychiatry and Psychotherapy, University of Tuebingen, Germany
3German Center for Neurodegenerative Diseases (DZNE), Tuebingen, Germany
4Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Germany

*These authors contributed equally to the study.

† Corresponding author:
Sebastian Heinzel
Psychophysiology & Optical Imaging
Department of Psychiatry and Psychotherapy, University of Tuebingen
Calwerstr. 14, 72076 Tuebingen, Germany
Phone: +49 7071 29 86125
Fax: +49 7071 29 4141
E-mail: Sebastian.Heinzel@med.uni-tuebingen.de
This work is part of the dissertation of S. Heinzel.
Subjects and Methods

Comorbidities within the ADHD patient group:

Of 181 adult ADHD patients included in the study 49.2% also had a current psychiatric comorbid axis I disorder as evaluated with the Structured Clinical Interview for DSM-IV (SCID-I) (Wittchen H et al., 1997):

22 patients were also diagnosed with substance misuse/dependency (alcohol abuse [F10.1; n=2] or dependency [F10.2; n=3]; cannabinoid abuse [F12.1; n=3] or dependency [F12.2; n=13]; stimulant dependency [F15.2; n=1]),

37 with mood disorders (bipolar affective disorders [F31.0, F31.8; n=6], depressive episodes [F32.1, F32.8; n=3], recurrent depressive episodes [F33.0, F33.1; n=8], cyclothymia [F34.0; n=3], dysthymia [F34.1; n=9], and other recurrent mood disorders [F38.1; n=8]),

30 with neurotic, stress-related and somatoform disorders (agoraphobia [F40.0; n=1], social phobias [F40.1; n=7], specific phobias [F40.2; n=7]; panic disorder [F41.0, F40.01; n=6]; generalized anxiety disorder [F41.1; n=3], obsessive compulsive disorder [F42.0; n=1], post-traumatic stress disorder [F43.1; n=3], bulimia nervosa [F50.2; n=1] and unspecified eating disorders [F50.9; n=1]).

Artifact criterions of electrophysiological data:

Three additional electrodes were attached at the outer canthi of both eyes and below the right eye for registration of eye movements. Data were corrected for ocular artifacts using the algorithm by Gratton and Coles (Gratton G and MGH Coles, 1989) and further epochs/segments were excluded if they contained any additional or remaining motor or technical artifact. In this regard, epochs containing artifacts (exclusion criteria: amplitudes > 70 µV or < -70 µV in any of the EEG-channels within
-100 ms to +700 ms relative to stimulus presentation [amplitude criterion]; or voltage steps > 70 µV from one sampling point to the next [gradient criterion]) were rejected. Only artifact-free epochs with correct behavioral responses were segmented and individually averaged to Go and NoGo event-related potentials (ERPs). The minimum number of trials averaged per participant and condition was n = 20.

**Supplement 2**

**Post-hoc tests**

**Post-hoc tests of COMT and DRD4 epistasis on Go-RT.**

One-way ANOVAs (dependent variable: Go-RT; between-subject factor COMT (number of Met-alleles)) and subsequent post-hoc tests were applied separately for the DRD4 genotype groups (“No 7R”, “7R”):

- **No 7R group:** $F_{2, 174} = 1.76; p = .18$
- **7 R group:** $F_{2, 115} = 2.94; p = .06$

Significant results of post-hoc tests:

- **7R group:** Go-RT (Val/Met-Met/Met): $t_{75} = 2.43; p = .02$

Comparisons (t-tests of independent samples) of Go-RT between DRD4 genotype groups (No 7R-7R); separately for COMT genotype subgroups:

- **COMT Val/Val:** $t_{80} = 1.02; p = .31$
- **COMT Val/Met:** $t_{128} = -2.16; p = .03$
- **COMT Met/Met:** $t_{81} = 1.74; p = .09$

**Post-hoc tests of COMT and DRD4 epistasis on Go-RT SD.**

One-way ANOVAs (dependent variable: Go-RT SD; between-subject factor COMT (number of Met-alleles)) and subsequent post-hoc tests were applied separately for the DRD4 genotype groups (“No 7R”, “7R”):
No 7R group: $F_{2, 174} = 4.46; p = .01$

7 R group: $F_{2, 115} = 1.90; p = .16$

Significant results of post-hoc tests:

No 7R group: Val/Val-Val/Met: $t_{120} = 2.61; p = .01$

Val/Met-Met/Met: $t_{134} = -2.47; p = .02$

7R group: Val/Met-Met/Met: $t_{75} = 1.73; p = .09$

Comparisons (t-tests of independent samples) of Go-RT SD between DRD4 genotype groups (No 7R-7R); separately for COMT genotype subgroups:

COMT Val/Val: $t_{80} = 96; p = .34$

COMT Val/Met: $t_{128} = -3.30; p = .001$

COMT Met/Met: $t_{81} = 1.12; p = .27$

Post-hoc tests of COMT and DRD4 epistasis on NGA.

One-way ANOVAs (dependent variable: NGA; between-subject factor COMT (number of Met-alleles)) and subsequent post-hoc tests were applied separately for the DRD4 genotype groups (“No 7R”, “7R”):

No 7R group: $F_{2, 174} = 3.96; p = .02$

7 R group: $F_{2, 115} = 3.58; p = .03$

Significant results of post-hoc tests:

No 7R group: Val/Val-Val/Met: $t_{120} = -2.72; p = .007$

Val/Met-Met/Met: $t_{134} = 2.05; p = .04$

7R group: Val/Val-Val/Met: $t_{88} = 2.57; p = .01$

Val/Met-Met/Met: $t_{75} = -1.68; p = .09$

Comparisons (t-tests of independent samples) of NGA between DRD4 genotype groups (No 7R-7R); separately for COMT genotype subgroups:
**COMT Val/Val**: $t_{80} = -2.26; p = .03$

**COMT Val/Met**: $t_{128} = 3.20; p = .002$

**COMT Met/Met**: $t_{81} = -.90; p = .37$

**Additional Analyses**

**Comparison of diagnostic groups**

Diagnosis did not significantly impact $DRD4 \times COMT$ epistasis on Go-RT SD ($p > .3$). Exploratory analyses revealed that the epistatic interaction described (inverted) U-shapes in ADHD patients as well as controls (see Supplementary Figure 1 a, b), however, controls with COMT Val/Met and $DRD4$ 7R genotype do not display increased Go-RT SD compared to corresponding ADHD patients ($t_{47} = 3.47; p = .001$).

**Supplementary Figure 1**
(a) Go-RT SD follows an (inverted) U-curve with increasing number of COMT Met-alleles in ADHD patients with DRD4 “no 7R” and “7R” genotype, respectively. (b) Healthy controls with “DRD4 no 7R” genotype also exhibit a U-relationship of Go-RT SD over COMT subgroups, however, in 7R carriers the inverted U-relationship is not present. Note that in the ANOVA (diagnosis x COMT x DRD4) diagnosis did not explain further variance regarding the COMT x DRD4 interaction. Exploratory (post hoc) analyses show that ADHD patients with COMT Val/Met and DRD4 7R genotype have a higher Go-RT SD compared to corresponding healthy controls (t_{47} = 3.47; p = .001). Error bars indicate standard error of the mean (SEM). Asterisks and asterisks in brackets indicate significant differences at a significance level of p < .05 and p < .10, respectively.

Similarly, DRD4 x COMT epistasis on NGA was not significantly impacted by diagnosis (p > .3), thus, (inverted) U-relationships are similar and post hoc
comparisons revealed no diagnosis-related differences in NGA (see Supplementary Figure 2 a, b).

Supplementary Figure 2

![Graph A](image1)

**ADHD patients**

- **DRD4 “no 7R” genotype**
- **DRD4 “7R” genotype**

![Graph B](image2)

**Controls**

- **DRD4 “no 7R” genotype**
- **DRD4 “7R” genotype**
(a) NGA describes an (inverted) U-curve over \textit{COMT} subgroups in ADHD patients with \textit{DRD4} “no 7R” and “7R” genotype, respectively. (b) Healthy controls with “\textit{DRD4} no 7R” genotype also exhibit (inverted) U-relationships of NGA over \textit{COMT} subgroups with \textit{DRD4} “no 7R” and “7R” genotype, respectively. Error bars indicate SEM.

References